

Original Research Article

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Seed Quality Parameters of Peanut and Soybean as Influenced by Seed Treatment with different Microbial Inoculants

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ABSTRACT

Keywords

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The cultivated species of Peanut (*Arachis hypogea*) and Soybean (*Glycine max*) belong to family Leguminosae. Leguminous crops have been cultivated since ancient times. Globally, Peanut and Soybean are the two most important oil-yielding leguminous crops. Yields of both Peanut and Soybean are lower in Asia than in developed countries. These low yields are due to a number of biotic and abiotic constraints. Hence, in order to increase crop yield per unit area, largely chemical fertilizers are used. The result of these activities in recent years has been the crisis of environmental pollution, especially water and soil pollution that threatens human society. Due to negative environmental impact of chemical fertilizer and their increasing costs, the use of soil microorganisms for sustainable agriculture has increased in various parts of the world. Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield. The PGPR used in this experiment are; *Phosphorous solubilizing bacteria* (PSB), *Trichoderma viride* and *Pseudomonas fluorescense*. The result of the study suggests that *Phosphorous solubilizing bacteria* (PSB) followed by *Pseudomonas fluorescense* retained maximum growth parameters in Peanut seeds. Whereas, in case of Soybean maximum growth parameters were observed in *Phosphorous solubilizing bacteria* (PSB) followed by *Trichoderma viride* treated seeds. This differential behavior in response of bio-fertilizers can be attributed to the different mode of phosphorus requirement and nitrogen fixation mechanism in respective crops.

Introduction

The cultivated species of Peanut (*Arachis hypogaea* L.) and Soybean (*Glycine max*. L.) belong to family Leguminosae. It is one of the largest and most useful plant family consisting of 19,327 species and 727 genera (Lewis, *et al.*, 2005) distributed throughout the world. Leguminous crops have been cultivated since

ancient times. India is blessed with the agro-climatic conditions favourable for growing nine major oilseeds including seven edible oilseeds, namely Peanut, Rapeseed, Mustard, Soybean, Sunflower, Safflower, Sesame and Niger and two non-edible sources, namely Castor and Linseed, apart from a wide range of other minor oilseeds and oil bearing tree species. Among all the oilseed crops, peanut

occupies the first place in India accounting more than 28% of acreage and 32% of production in the country. Globally, Peanut and Soybean are the two most important oil-yielding leguminous crops. Peanut or Groundnut (*Arachis hypogaea* L.) which is also known as a 'King' of oilseed (Sathya Priya *et al.*, 2013) is a rainfed crop in kharif season and irrigated crop in rabi in some states (Varghese, 2011). It is rich in protein and vitamins A, B and its calorific value is 349 K cal. per 100 gram seed. It is grown over an area of 5.31 million ha and producing 6.93 million tonnes of Peanut (DOAC, 2012) with productivity of 1305 kg/ha in Indian context. Its cultivation is mostly confined to the states of Gujarat, Andhra Pradesh, Maharashtra, Tamil Nadu and Karnataka. Peanut contributes about 40 per cent of the total oilseeds production in the country (Sathya Priya, *et al.*, 2013).

Soybean also called "edible grain legumes" can be divided into two types: oilseeds and pulses. Together, Soybean oil and protein content account for about 60 per cent of dry Soybeans by weight (protein 40% and oil 20%). It is the second only to Peanut in terms of oil content (20%) among food legumes (Bekere and Hailemariam, 2012). Soybean has an important place in world's oilseed cultivation scenario, due to its high productivity, profitability and vital contribution towards maintaining soil fertility. The crop also has a prominent place as the world's most important seed legume, which contributes 25% to the global vegetable oil production.

Peanut also has value as a rotation crop because through root nodules, it can symbiotically fix atmospheric nitrogen and therefore improves soil fertility. Yields of both Peanut and Soybean are lower in Asia than in developed countries. These low yields are due to a number of biotic and abiotic constraints,

such as farmers' lack of access to quality inputs, improved technologies and information and frequent attacks by pests and diseases. Hence, in order to increase crop yield per unit area, largely chemical fertilizers are used. The result of these activities in recent years has been the crisis of environmental pollution, especially water and soil pollution that threatens human society.

Sustainable agriculture based on using biological fertilizers is an effective solution for overcoming these problems. Biological fertilizers contain useful enzymes and microorganisms that can increase plant growth and quality of crops, and reduce the cost of fertilizer and pesticides application. Phosphate-solubilizing microorganisms produce various organic acids such as oxalate, lactate, acetate, glycolate, gluconate, tartrate, etc. It has been reported that the addition of bio-agents, both fungal and bacterial induced the growth of various crop plants (Khan, 2005). These organisms also provide protection against diseases by suppressing deleterious and pathogenic microorganisms. *Trichoderma spp.* are effective in control of soil/seed borne fungal diseases in several crop plants (Kubicek *et al.*, 2001), including groundnut (Podile and Kishore, 2002).

Nodules formed by the strains may not be able to fix sufficient nitrogen to meet the demand of the plant. Phosphorus plays an important role in nodulation of legume crops. Phosphobacterium, a *Phosphate solubilising bacteria*, able to convert the unavailable phosphate present in the soil to make it available to the plant, has an indirect but definite effect on the nodulation and yield of legume crops like Peanut and Soybean. *Phosphate solubilising bacteria* improve nodulation (Ghosh and Poi, 1998) through increased phosphate solubilisation and hence, increase symbiotic nitrogen fixation.

Materials and Methods

Material collection

Samples of certified seeds of Peanut (*Arachis hypogaea* L.) variety JGN-23 and Soybean (*Glycine max* L.) variety JS-9560 were collected from Krishi Vigyan Kendra (KVK) Ujjain (M.P.) and the bio fertilizers used includes; *Phosphorus solubilizing bacteria* (PSB), *Trichoderma viride* and *Pseudomonas fluorescense* were collected from Indore Inputs and Research Pvt. Ltd.

Treatment details

The details of the microbial (bio-fertilizer) seed treatments used in this experiment are furnished below:

T₁: *Phosphorus solubilizing bacteria* (PSB) @ 15 g/ Kg compost and 5g/ Kg of seeds.

T₂: *Trichoderma viride* @ 15 g / Kg of compost and 5g / Kg of seeds.

T₃: *Pseudomonas fluorescense* @ 15 g / Kg of compost and 5g / Kg of seeds.

T₄: Untreated Control

Experimental design

Completely randomized design (RBD) with three replication for each Microbial (Bio-fertilizer) seed treatments.

Procedure of microbial (Bio-fertilizer) seed treatments

The experiment was arranged in a randomized block design consisting of four microbial (Bio-fertilizer) seed treatments and each microbial seed treatment contains three replications. These microbial seed treatments were thoroughly mixed with compost in a bucket @ 15g / Kg of compost. About 300 seeds were planted for each treatment in three replicated pro trays. These pro trays were placed greenhouse net and these pro trays were

regularly giving water spray. Seeds were observed for germination in all the set ups every day.

Statistical analysis

One way ANOVA using CRD design and online analysis carried out by using OPSTAT.

Bio-matric observations

Germination test

Germination test was done on field performance evaluation along with pro trays filled with compost and coco-pit and about 100 seeds were planted at 1cm depth at an equal spacing.

Shoot and root length (cm)

Fifteen normal seedlings were selected randomly from three replicates of each treatment for the measurement of shoot and Root length. The shoot length was measured from the collar region to the tip of the primary leaf and the root length was measured from the collar region to the tip of the primary root.

Seedling dry weight (mg)

Fifteen normal seedlings used for measuring of shoot and root were also used to determine seedling dry weight. The seedlings were kept in butter paper bags and dried in a hot air oven maintaining at 70⁰C for 24 hrs. and after completion of that then cooled in a desiccators for 30 minutes, the weighing was done in an electric balance. The weight of dried samples was recorded and average of fifteen seedling dry weight in milligrams was recorded.

Seedling Vigor Index (SVI)

The vigour index of seedling was calculated by adopting the method suggested by Abdul-baki and Anderson (1973).

SV-I = Total germination% x Total seedling length (cm)

SV-II = Total germination % x Total seedling dry weight (mg)

Chlorophyll content (mg/g)

Chlorophyll content of the leaves of selected plants was estimated by Arnons method (1949). The amount of chlorophyll was calculated by using the following formula;

$$\frac{\text{Total Chlorophyll (mg/g)}}{20.2 \times A_{645} + 8.02 \times A_{663}} \times V = \alpha \times 1000 \times \omega$$

$$\text{Chlorophyll a (mg/g)} = \frac{12.7 \times A_{663} - 2.69 \times A_{645}}{\alpha \times 1000 \times \omega} \times V$$

$$\text{Chlorophyll b (mg/g)} = \frac{22.9 \times A_{645} - 4.68 \times A_{663}}{\alpha \times 1000 \times \omega} \times V$$

Where,

A = Absorbance at 645nm and 663nm.

α = Length of light path in the cuvette.

V = Volume of the extract in ml.

ω = fresh weight of the sample.

Leaf area (cm²)

Leaf area was calculated as per Hoyt and Brandfield (1962) by using the following equation as shown below;

$$LA = LL \times LB \times 0.75$$

Where;

LA is the leaf area

LL is the leaf length

LB is the leaf breadth

0.75 is the correction factor for the leaf shape

Results and Discussion

The data on different seed quality parameters of Peanut variety JGN-23 and Soybean variety JS-9560 seeds treated with different microbial (Bio-fertilizers) treatments. The detailed explanation of the study is as follows;

Bio-metric observation

According to the results of variance analysis PGPR isolates significantly enhance different seed quality parameters (Germination percentage, shoot length, Root length, total seedling length and seedling dry weight) of Peanut and Soybean seedlings over control. However, the rate of enhancement varied with bacterial strains.

Data from Table 1 observed that highest seed quality parameters in Peanut was observed in seeds treated with *Phosphorus solubilizing bacteria* T₁ (PSB) followed by *Pseudomonas* T₃ and *Trichoderma viride* T₂ respectively, and the lowest germination percentage was recorded in control T₄. Similarly, in case of Soybean the highest seed quality parameters was observed in seeds treated with PSB T₁ followed by seeds treated with *Trichoderma viride* T₂ and *Pseudomonas* T₃ and the lowest seed quality parameters was recorded in control T₄ as mentioned in the Table 2.

The increment of seed quality parameters with inoculants could be due to the isolates ability to synthesize seed germination hormone like gibberellins which triggered the activity of specific enzymes that promote early germination, such as α - amylase that increase the availability of starch for assimilation. It could also be a result of better activity of mitochondrial enzymes accompanied by an increase of the oxygen consumption.

Seedling Vigour Index SVI-I (mg) and Seedling Vigour Index SVI-II (mg)

The highest seedling vigour index-I and seedling vigour index-II was recorded in seeds treated with *Phosphorus solubilizing bacteria* (PSB) T₁ followed by seeds treated with *Pseudomonas* T₃ and *Trichoderma viride* T₂. Whereas, lowest seedling vigour index-I and seedling vigour index-II were recorded in control seeds T₄ Table 1 .

In case of Soybean the highest seedling vigour index-I and seedling vigour index-II was recorded in seeds treated with PSB T₁ followed by seeds treated with *Trichoderma viride* T₂ and *Pseudomonas* T₃ with significant difference between them. Lowest seedling vigour index –I and seedling vigour index-II was recorded in control seeds Table 2.

Non-treated seeds with bio-fertilizer could cause decrease of seed vigour index, it seems that treated seeds with bio-fertilizer transfer nutrition material efficiently to embryo results in improved growth of root and shoots lengths and increased SVI. This high vigour index may be due to a better production and metabolism of auxin, responsible for cellular elongation or cytokinin, hormone that stimulate the cellular division triggered by PGPR treatments.

Leaf area (Cm²)

Results observed that seeds of both crops treated with these PGPR treatments shows

increase in leaf area as compared to the seeds of untreated control one. In Peanut highest leaf area was recorded in seeds treated with *Phosphorus solubilizing bacteria* (PSB) T₁ (1.5 cm²) followed by seeds treated with *Pseudomonas* T₃ (1.32cm²) and *Trichoderma viride* T₂ (1.30cm²) with significant differences between T₁ and T₃ and the lowest leaf area index was recorded in control seeds T₄ (1.2 cm²) without any significant differences between them (Table 1). In Soybean the highest leaf area was recorded in seed treated with *phosphorus solubilizing bacteria* (PSB) T₁ (8.24 cm²) followed by *Trichoderma viride* T₂ (6.29 cm²) and *Pseudomonas* T₃ (5.79 cm²) with significant difference between them and lowest leaf area was recorded in control seeds T₄ (5.29 cm²) as given in Table 2. Leaf area index and dry biomass yield increased with the increase in P solubilization and P uptake due to the influence of PSB. The increase in leaf area enhances photosynthesis rate and this enhancement leads to increase in yield.

Table.1 Effect of different microbial inoculants on germination %, shoot and root length, total seedling length, seedling dry weight, vigour index and leaf area of peanut (*Arachis hypogaea* L.)

Treatments (T)	Germination %	Shoot Length (cm)	Root Length(cm)	Total seedling Length(cm)	Seedling dry Weight. (mg)	SV-I	SV-II	Leaf area (cm ²)
T ₁	78.5	10.8	7.6	18.4	0.25	1506	18.7	1.517
T ₂	73.4	10.1	6.5	16.1	0.240	1139	16.0	1.30
T ₃	76.5	10.2	6.6	16.7	0.247	1187	16.4	1.32
T ₄	71.4	9.8	5.8	15.6	0.22	1019	14.4	1.28
C.D.	1.239	N/A	N/A	1.880	N/A	135.148	1.433	N/A
S.Em.±	0.351	0.232	0.507	0.533	0.024	38.31	0.40	0.186

Table.2 Effect of different microbial on germination %, shoot and root length, total seedling length, seedling dry weight, vigour index and leaf area of soybean (*Glycine max* L.)

Treatments (T)	Germination%	Shoot Length(cm)	Root Length(cm)	Total seedling Length(cm)	Seedlings dry Weight. (mg)	SV-I	SV-II	Leaf area(cm ²)
T ₁	83	14.16	8.73	22.5	0.10	2066	8.28	8.24
T ₂	82.8	12	8.00	20.1	0.09	1929	8.0	6.29
T ₃	81.9	11.6	7.53	19.1	0.06	1820	6.7	5.79
T ₄	79.3	9.7	6.16	15.4	0.05	1296	3.3	5.29
C.D..	0.493	0.640	0.783	0.473	0.019	69.864	0.626	N/A
SE(m)	0.140	0.181	0.222	0.134	0.005	19.804	0.177	0.638

Table.3 Effect of different microbials on chlorophyll contents of peanut and soybean

Treatments (T)	Peanut (<i>Arachis hypogaea</i> L.)			Soybean (<i>Glycine max</i> L.)		
	Chlorophyll a (mg / g)	Chlorophyll b (mg / g)	Total Chlorophyll (mg / g)	Chlorophyll a (mg / g)	Chlorophyll b (mg / g)	Total Chlorophyll (mg / g)
T1	0.4652	0.3788	0.8483	0.4624	0.3238	0.7858
T2	0.4384	0.3163	0.7546	0.4284	0.2467	0.6752
T3	0.4648	0.3780	0.8430	0.4267	0.2362	0.6628
T4	0.4017	0.2544	0.6562	0.4185	0.2290	0.6470

Where: T₁=*Phosphorus Solubilizing bacteria* (PSB).T₂ =*Trichoderma viride*.T₃ = *Pseudomonas fluorescense*T₄ = Control (Untreated)

Figure.1 Effect of different microbials on chlorophyll contents of peanut

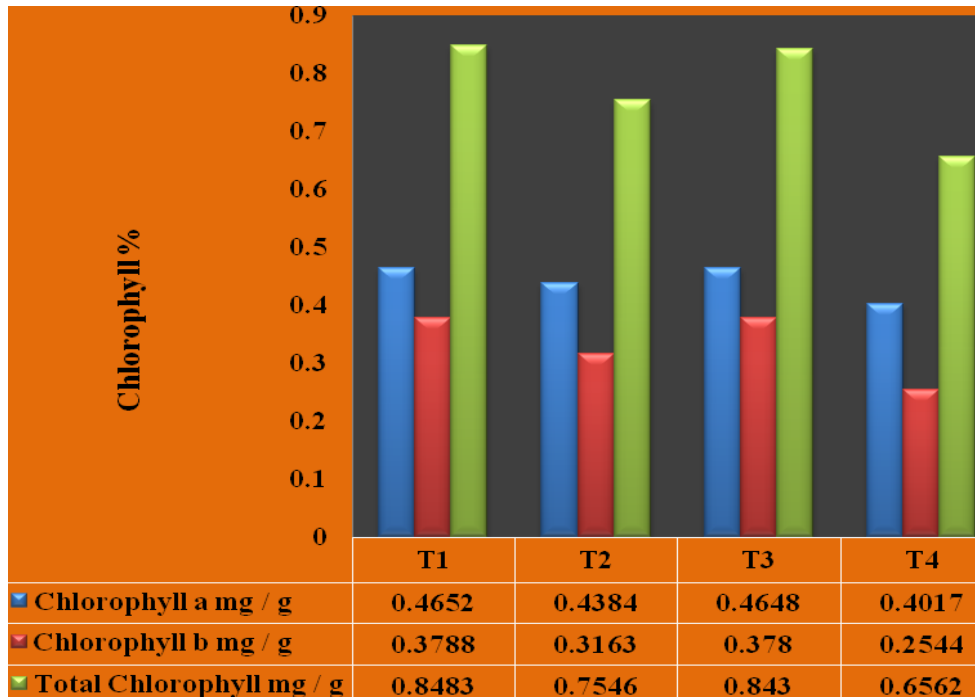
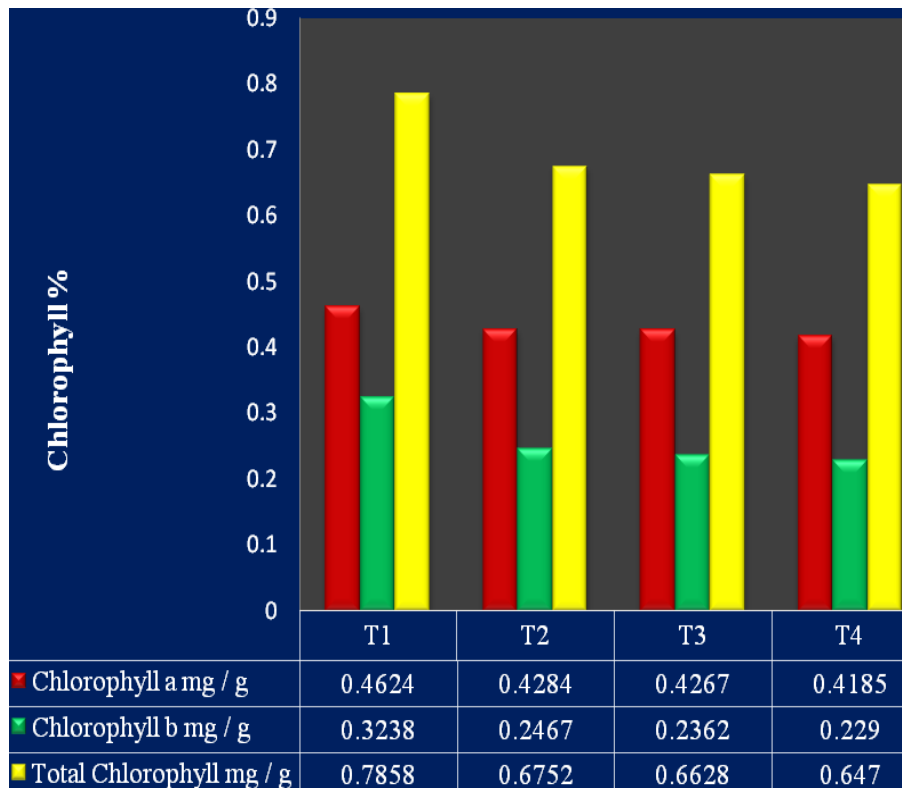


Figure.2 Effect of different microbials on chlorophyll contents of soybean



Chlorophyll content (mg/g)

The result of the experiment described that chlorophyll content of peanut and Soybean was significantly influenced by PGPR treatments. Data mentioned in Table 3 revealed that maximum chlorophyll content of Peanut viz., chlorophyll 'a', chlorophyll 'b' and total chlorophyll were recorded in seeds treated with *Phosphorus solubilizing bacteria* (PSB) T₁ (0.4652mg/g, 0.3788mg/g and 0.8483mg/g), which was at par with seeds treated with *Pseudomonas* T₃ (0.4648 mg/g, 0.3780mg/g and 0.8430mg/g) and seeds treated with *Trichoderma viride* T₂ (0.4384mg/g, 0.3163mg/g and 0.7546mg/g). Whereas, minimum chlorophyll content was recorded in control seeds T₄ (0.4017mg/g, 0.2544mg/g and 0.6562mg/g). Similarly, in Soybean data mentioned in the Table 3 stated the maximum chlorophyll content viz., Chl. a, Chl. b and total chlorophyll were recorded in seeds treated with *Phosphorus solubilizing bacteria* (PSB) T₁ (0.4624mg/g, 0.3238mg/g and 0.7858mg/g) followed by seeds treated with *Trichoderma viride*T₂ (0.4284mg/g, 0.2467mg/g and 0.6752mg/g), which was at par with the results of seeds of *Pseudomonas* T₃ (0.4267mg/g, 0.2362mg/g and 0.662 mg/g). Whereas minimum chlorophyll content was recorded in seeds of untreated control T₄Chl.a (0.4185 mg/g), Chl.b (0.2290 mg/g) and total chlorophyll (0.6470 mg/g). N-Fixing PGPR is able to supply high amount of nitrogen for tissue growing and therefore increases chlorophyll content. PSB inoculated treatments increased leaf chlorophyll values and resulted in higher leaf photosynthesis compared to non-inoculated treatments (Fig. 1 and 2).

In conclusion, the result of the study suggests that Phosphorus solubilizing bacteria (PSB) followed by *Pseudomonas* fluorescence retained maximum growth parameters in Peanut seeds. Whereas, in case of Soybean

maximum growth parameters were observed in Phosphorus solubilizing bacteria (PSB) followed by *Trichoderma viride* treated seeds. This differential behavior in response of bio-fertilizers can be attributed to the different mode of phosphorus requirement and nitrogen fixation mechanism in respective crops. It is also suggested that applications of all microbial (bio-fertilizer) seed treatments did not affect any of the crops adversely and proved to be beneficial for observing maximum quality parameters over control due to their inherent capacity to produce plant growth promoting substances. In certain conditions they also exhibit antifungal activities and there by fungal disease may be controlled indirectly. The use of these bacteria strains offers a way to reduce chemical fertilizers doses. Increasing and indiscriminate use of chemical fertilizer may affect soil health and may lead to a negative impact on soil fertility by destroying so many microorganisms which are beneficial for increasing soil fertility. Hence for sustainable agriculture, bio-fertilizer is most important for agricultural purposes. Under the changing agricultural scenario, the only technology that seems promising to enhance seed quality parameters without disturbing the equilibrium of harmful and useful composition of environment and ecosystem is the use of more and more biological control agents or bio-fertilizers.

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